IN THE SPECIFICATION

At page 1, line 3, insert the following new paragraph:

BACKGROUND OF THE INVENTION

At page 1, line 24 insert the following new paragraph:

SUMMARY OF THE INVENTION

At page 8, line 23 insert the following new paragraphs:

BRIEF AND DETAILED DESCRIPTION OF THE DRAWINGS

Brief Description of the Figures

Fig. 1 discloses an embodiment for proximity-dependent selection.

Fig. 2 discloses different approaches for accomplishing coupling.

Fig. 3 discloses four different approaches for producing a coupling.

Fig. 4 discloses a library versus library screening.

Fig. 5 discloses an embodiment in which a target oligonucleotide association is performed in solution.

Fig. 6 discloses a target oligonucleotide association on cell surface.

Fig. 7 discloses a target with multiple binding sites which may associate with members of the bifunctional molecule library.

Fig. 8 discloses a target with one binding site for association

with a pair of displayed molecules.

Fig. 9 discloses a 2nd generation-library driven proximity selection.

Fig. 10 discloses multiple targets for simultaneously subjected to a library of complexes.

Fig. 11 discloses two embodiments of using a Taqman probe (5'' nuclease probe) in the measurement of the presence or absence of a certain codon.

Fig. 12 discloses a standard curve used in example 3.

Fig. 13 discloses the result of two experiments reported in example 3.

Detailed Description of Figures 1-10

Fig. 1 outlines an embodiment for a proximity-dependent selection (PDS). The molecular target is linked to a target oligonucleotide, which in some embodiment may be unique for the target molecule. This target sequence comes in close proximity with a specific identifier oligonucleotide when the displayed molecule of a bifunctional complex binds to the target molecule. This proximity will promote the coupling between the bifunctional complex molecules that bind to the target compare to bifunctional complex in solution. Thus, there will be a selection for coupling products that contain display molecules that possess affinity for the target molecule. The final ligation product is amplified using two primers that only amplify ligated products.

In a first step, the target associate with a target oligonucleotide is mixed with a library of complexes, in which each complex comprises a display molecule attached to an

identifier oligonucleotide. The display molecules are then incubated with the target. The display molecules which have an affinity towards the molecular target will bind, while the complexes not having affinity will remain in solution. Subsequent to the incubation, a connector oligonucleotide is added. The connector oligo nucleotide comprises parts that hybridise to sequences near the ends of the target and the identifier oligonucleotides, respectively. Subsequent to the addition of the connector oligonucleotide, a ligation is effected by chemical or enzymatic means. Preferably a ligase is used to ligate the target and the identifier oligonucleotides together. The connector oligonucleotide is generally added in excess to saturate the complexes in solution to avoid unspecific ligation.

After the ligation, the ligation product is amplified by PCR. Thus, a forward primer is annealed to the ligation product at the 3'' end thereof and extended using a polymerase. The transcribed product comprises a site to which a second (or reverse) primer can anneal so as to provide for an extension of the second primer using the transcribed product as template. Using forward and reverse primers as indicated above together with a polymerase and suitable substrates produces amplicons, which comprises information about the display molecule as well as the molecular target. The ligated product can be introduced into a host organism using a suitable vector. The host vector may be allowed to form colonies and the colonies can be sequenced to establish the identity of the display molecule.

Fig. 2 shows various options to perform coupling between the target oligonucleotide and the identifier oligonucleotide. A. The ligation is promoted using a connector oligonucleotide that anneals both to the target oligonucleotide and the identifier oligonucleotide. The connector oligonucleotide is designed such that the ends of the identifier oligonucleotide and the target oligonucleotides are abutted. A ligase is subsequently allowed

to ligate the ends together. B. A connector oligonucleotide is used to promote fill in of a gap using a polymerase and finally ligation using a ligase. C. The distal end of the target oligonucleotide overlaps the distal end of the identifier oligonucleotide, which allows a polymerase to extend the target oligonucleotide as well as the identifier oligonucleotide thereby forming a double stranded product. D. Blunt-ended ligation of single-stranded or double stranded DNA using a suitable enzyme like T4 DNA ligase.

Fig. 3 shows various methods for preparing a coupling area on an existing bifunctional complex. Conjugates between molecular targets associated with an oligonucleotide and complexes comprising a display molecule and an identifier oligonucleotide can be modified to allow a ligase to couple the oligonucleotides together. A. The identifier oligonucleotide is extended with a primer with an overhang that creates the coupling area. The extension is suitably conducted before the selection process to obtain the benefit of a double stranded nucleotide sequence. A target oligonucleotide can be ligated to the blunt end of the extended primer or a connector oligonucleotide can be used to connect the target oligonucleotide and the extended primer prior to ligation with a suitable ligase. B. The identifier oligonucleotide is annealed to a primer that binds internally. The primer is subsequently extended, suitably before the selection process. The extension forms a coupling area directly on the identifier oligonucleotide, which allows a target oligonucleotide to be annealed and ligated. C. The first step is identical to the procedure as describe in B but the target sequence has a free 5''-end that allow ligation to the 3''-end of the identifier oligonucleotide. A blunt ended single stranded ligation can be performed. Alternatively, this variation can be performed using a connector oligonucleotide and subsequent ligation. D. A primer is annealed to a identifier oligonucleotide and extended to produce a double-stranded DNA which is

subsequently cut with an enzyme (e.g. restriction enzyme) to produce a single-stranded DNA portion that can be used as handle in the coupling process.

Fig. 4 shows a library versus library selection method. Different targets specifically encoded by the attached target oligonucleotides are mixed with a library of bifunctional complexes. The displayed molecules will bind to specific targets and promote the ligation through the proximity effect. This ligation will connect the target oligonucleotides with oligonucleotides that encodes for specific displayed molecules. The ligated oligonucleotides can be amplified and determined by sequencing procedures well known in the art. The ligated sequences will reveal which display molecules that bind to which target.

Fig. 5 discloses inter alia the association of the target oligonucleotide to the target. One way of associating the target oligonucleotide with the target molecule is to link the oligonucleotide through a tag introduced on the target molecule. The tag can be attached before the target is produced (e.g. a short amino acid sequence such as HIS-tag of FLAG-tag) or be modified after the target is produced. The target sequence can then be associated through the tag using a tag-binding molecule such as an antibody or other type of molecules that binds to the tag.

Fig. 6 discloses target oligonucleotide association on a cell surface. Specific receptors can be engineered to express a specific tag on the cell surface. Different tags can be used such as HIS- or FLAG-tags or other types of tags that become bound with the receptor. The tag will only be displayed on the cell surface together with the specific receptor. The target oligonucleotide is then associated with the receptor target using a mediator molecule that carries the target oligonucleotide and

binds to the tag. A mediator molecule could be an antibody that binds to the tag (e.g. anti-HIS or anti-FLAG antibodies) that is associated with the target oligonucleotide. This procedure will specifically associate the target oligonucleotide with a receptor target on the cell surface which will promote a ligation between oligonucleotides of the binding displayed molecules and the target oligonucleotide.

Fig. 7 shows a target molecule with several sites for binding of ligands. The target is subjected to a library of complexes of bifunctional molecules. Display molecules of the complexes binds to the discrete sites of the molecular target thus promoting a high local concentration of the ends of the oligonucleotides which have bound to the target. Subsequently a connector oligonucleotide is added to adjoin the distal ends of the oligonucleotides together. Usually, the connector oligonucleotide is added in excess to saturate the ends of the identifier oligonucleotides free in the solution. The ends of the oligonucleotides kept together by the connector oligonucleotide are ligated together forming a coupled product. The coupled product is amplified by PCR using primers annealing to each end of the coupled product. The amplified coupled product is decoded to identify the display molecules which have bound to the in the target. In a step not shown on the figure, the two binding display molecules are coupled together via a suitable linker to form a ligand which binds to two sites of the target. Suitable, the dimer comprising the two revealed display molecules and the linker is synthesised by organic synthesis.

Libraries of bifunctional complexes can also be screened against each other using the present invention. Such an embodiment allows the detecting of pairs of displayed molecules that bind to the same target at different or the same binding site or pair of displayed molecules that bind to different targets. The power of the screening libraries in the above fashion is indicated by the

fact that a library of e.g. 10^4 different displayed molecules generates a total combination of display molecules of 10^8 when pair of binders are searched for.

Fig. 8 discloses a library of bifunctional complexes which is presented to a target having a site possible to be occupied by two display molecules. Initially, the target is mixed with the library of bifunctional complexes under conditions which promote a binding interaction to take place. A first bifunctional complex associates with the target to form the target associated with the target oligonucleotide. Subsequently to or simultaneously with the binding of the first display molecule, a second complex binds to the same site of the target. The display molecules may or may not be reacted with each other to form a covalent linkage between the display molecules. In another embodiment, the two display molecules are connected via a suitable linker or reacted with an external reactant so as to form a single molecule. After the binding interaction of the library of complexes with the target, the ends of the complexes which comprises display molecules that binds to the target are joint together. In an aspect of the invention, the ends are joined together using a connector polynucleotide. The connector polynucleotide is preferably added in excess to saturate ends of identifier oligonucleotides which are not part of a binding complex. After the hybridisation event between the ends of the identifier oligonucleotides and the connector oligonucleotide a ligation is conducted. Suitably the ligation is performed by a ligase to form a coupled product, which can be used as a template by a polymerase. After the ligation, the coupled product is amplified by PCR to form PCR amplicons comprising information of the display molecules which have participated in the binding interaction.

Fig. 9 discloses a two (or more) step identification method. In a first step the method as disclosed in figure 1 is conducted and in the second step a new library prepared upon the knowledge

harvested in the first library is used to generate the second generation library. Initially, a library of complexes are is presented to a target having a binding site. In the library, display molecules having a binding affinity above a certain threshold is not present, illustrated on the drawing with a display molecule only having a partial fit in the binding site of the target. In the synthesis of the second generation library components used in the synthesis of the low binding display molecule are shuffled with further components and/or the low binding display molecule is added or subtracted a structural unit. As an example, a further round of addition of chemical entities can be conducted. For systems for complex generation relying on the natural translation system a deletion, alteration or addition of nucleic acid can be performed. The second generation library is presented to the a target again. In the event the alteration of the initial low binding molecule has been successful display molecules are generated which binds with a higher affinity towards the target.

Fig. 10 discloses two targets which are attached to each other prior to the mixing with the library of complexes. The attachment can be natural, i.e. the association between target 1 and target 2 occur in a biological context or the attachment can be artificial, i.e. the association between target 1 and target 2 is obtained by a chemical synthesis. In the latter instance, the association between the targets may be obtained by any chemical or enzymatic means which ensure a linkage. The association of the targets may also be obtained by expressing target 1 and target 2 as a fusion protein, i.e. a single protein having two distinct targets or monomer domains. In an embodiment, one of the targets in the fusion protein is a capturing protein, like streptavidin. In the event the library is spiked with complexes having a ligand against the capturing protein, like biotin, it is feasible to form a connection between the fusion protein and a member of the library. The further functionality, i.e. target 2, of the fusion

protein may be then be subjected to a screening process to find binder from the library.

During the mixing step, the two attached targets are contacted with the library of complexes under binding conditions. The library may be spiked with a complex comprising a compounds known to bind to the one of the targets in order to find suitable binders against another target. If the library comprises suitable binding display molecules, two ends of the binding complexes is positioned in close proximity. The addition of a connector oligonucleotide ensures that the ends are kept close together when a ligase is allowed to perform the action of ligating the ends together. The resulting PCR product comprises genetic information which encodes both the display molecules that have participated in the binding interaction with target 1 and target 2.

Please delete the paragraphs at P112, L22 to 119, L17 and at P43, L17-23.